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# VISUAL PATHWAYS AND THE CENTRAL NEURAL CONTROL OF A CIRCADIAN RHYTHM IN PINEAL SEROTONIN N-ACETYLTRANSFERASE ACTIVITY

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SUMMARY

The pineal enzyme, serotonin N-acetyltransferase, exhibits a circadian rhythm of activity with nocturnal levels 15–30 times greater than those observed during a light period in the rat. This rhythm has been shown to be under visual control mediated by the sympathetic innervation to the pineal. The present study examined the participation of visual pathways and other central mechanisms in the regulation of pineal serotonin N-acetyltransferase activity. Following destruction of all visual pathways by blinding, the rhythm in enzyme activity is no longer controlled by the pattern of diurnal lighting and becomes free-running. Destruction of the primary optic tracts, the accessory optic tracts, or both of these components of the central retinal projection together, does not alter visual entrainment of the enzyme rhythm. In the absence of these pathways the only central retinal projection known to exist is a retinohypothalamic pathway branching directly off the optic chiasm to terminate bilaterally in the suprachiasmatic hypothalamic nuclei. Selective ablation of these nuclei, sparing the optic chiasm, abolishes the circadian rhythm in pineal serotonin N-acetyltransferase. This effect is mimicked by a knife cut across the medial hypothalamus caudal to the suprachiasmatic nuclei and by bilateral lesions transecting the medial forebrain bundle within the lateral hypothalamus, but a hypothalamic knife cut just rostral to the nuclei has no effect upon the rhythm. It is concluded that the retinohypothalamic projection to the suprachiasmatic nuclei is essential for maintaining the entrainment to light of the circadian rhythm in pineal serotonin N-acetyltransferase activity in the rat. In addition, the observations presented here suggest that the suprachiasmatic nuclei represent a central rhythm generator having projections directed caudally into the medial hypothalamus and into the medial forebrain bundle in the lateral hypothalamus.

## INTRODUCTION

The pineal gland once again has become a fashionable object of scientific investigation and there is now substantial evidence that it is an endocrine organ with antigonadal activity[25,26]. One substance elaborated by the pineal gland which has antigonadal activity in some species is melatonin. Melatonin is a major product of the very active indoleamine metabolism system in the pineal gland[2,3,4,12]. The amount of melatonin in a pineal gland varies with a diurnal rhythm[16,24]. Recent studies[12,14] indicate that melatonin synthesis[1–3] in the pineal gland is regulated by the activity of the enzyme which converts serotonin to N-acetylserotonin, serotonin N-acetyltransferase (EC 2.3.1.5)[32,33], which also exhibits a diurnal rhythm[12,14]. Like the rhythm in melatonin content[16,24], the pineal N-acetyltransferase rhythm is influenced by the pattern of environmental lighting. N-acetyltransferase activity is low during the light period and increases during darkness to reach levels 15–30-fold greater than observed during a light period[12–14]. The N-acetyltransferase rhythm has the characteristics of a true circadian rhythm in that it becomes free-running in the absence of visual cues, is abolished by continuous light, and is precisely entrained by the pattern of diurnal lighting[12–14]. At the present time there is little information available about the visual pathways or other central neural mechanisms that control circadian rhythms, either in the pineal or in other aspects of mammalian functions[19]. The experiments reported here were designed to investigate one aspect of this problem by determining the specific central neural structures mediating the generation of the pineal N-acetyltransferase rhythm and its control by diurnal light.

## MATERIALS AND METHODS

The subjects used in the experiments of this study were female albino rats of the Holtzman strain (Holtzman Co., Madison, Wisc.) obtained at 180–200 g body weight. Throughout the experiment the animals were housed in groups of 6–8 in clear plastic cages covered with a wire grill. The cages were on racks within a room illuminated by Vita-Lite (DuroTest Corp., Chicago, Ill.) fluorescent bulbs. The intensity was approximately 50 ft. candles of light within the cages during the lighting period. Diurnal lighting was automatically controlled so that lights were on from 0700 to 1900 h each day. The animals were conditioned to the laboratory and lighting schedule for at least 10 days prior to any surgical procedure. They had free access to food and water at all times.

### *Surgical and histological procedures*

For each surgical procedure the animals were anesthetized with anesthetic ether, placed in a rat stereotaxic apparatus (Kopf Instrument Co., Tujunga, Calif.), operated upon, given 20 mg oxytetracycline intramuscularly, removed from the stereotaxic apparatus, allowed to recover, and returned to the home cage. Three separate sets of experiments were performed and a group of sham operated animals were prepared

with each experiment (Figs. 6–8). Prior to operation for each experiment the animals were divided into groups of predetermined size so that each operated group would contain sufficient numbers to allow no less than 6 animals to be sacrificed at each of 4 time points around the clock. In prior studies[9,21,22] medial forebrain bundle lesions, primary optic tract lesions, and combined primary and accessory optic tract lesions had been found to incur approximately a 50–60 % postoperative mortality and this was taken into account in planning the size of these groups before operation. Similarly, we found that a large number of operated animals were required to provide a sufficient number with suprachiasmatic lesions that ablated the suprachiasmatic nuclei bilaterally but spared the optic chiasm[20]. All animals were allowed to survive for 21 days after operation and were then sacrificed by decapitation in one 24-h period at the following times: 0700, 1300, 1900 and 2400 h. The pineal glands were rapidly removed and frozen individually on dry ice. The brains from animals with central lesions were removed, fixed in 10 % formalin, and prepared for histologic confirmation of lesion placement. A few days prior to sacrifice the animals with visual pathway lesions and hypothalamic knife cuts were observed for gross visual responses and pupillary responses to light. Individual operative procedures were carried out as follows.

*Sham operation.* The skin was incised and a burr hole placed in the skull. As with the following procedures, the incision was closed with wound clips.

*Blinding.* Bilateral orbital enucleation was performed by dissection through the periorbital tissues, cutting the optic nerve at its entrance into the globe, and removing each eye.

*Accessory optic tract lesions.* Bilateral transection of the accessory optic tracts was performed by removing one eye and placing an electrolytic lesion in the ipsilateral optic tract just at its exit from the optic chiasm. The coordinates for this lesion were anterior 7.0 mm from ear bars, lateral 1.2 mm, and ventral 2.5 mm below horizontal zero with the tooth bar 5 mm above the ear bar. An anodal DC current of 2 mA was passed for 30 sec with the cathode attached to the skin of the back. The accessory optic tracts are completely crossed in the rat. Therefore, this combination of lesions will produce their complete, bilateral destruction.

*Primary optic tract lesions.* Large, bilateral electrolytic lesions were placed in the lateral geniculate nuclei to totally transect the optic tracts at their entrance into the thalamus as described previously[21].

*Primary and accessory optic tract lesions.* Bilateral lesions of both retinal projections were made by combining the lesions described above in the following way. One eye was removed, the ipsilateral optic tract was destroyed at its emergence from the chiasm and the contralateral optic tract was destroyed at the level of entrance into the lateral geniculate nuclei. With this combination of lesions the only intact fibers leaving the optic chiasm were those of the retinohypothalamic tract originating in the remaining eye.

*Prechiasmatic cut.* The animal was placed in the stereotaxic apparatus with tooth bar at zero, the sagittal sinus was exposed and incised and a modified Halasz knife[8] (the vertical and horizontal components 2.4 mm in length) was stereotaxically lowered through the midline at a level 1 mm rostral to the bregma until it touched the

skull base. It was rotated in a semicircle and then removed vertically through the midline.

*Suprachiasmatic lesions.* Bilateral electrolytic lesions were placed in the suprachiasmatic region using the coordinates and other parameters described previously[20].

*Postchiasmatic cut.* This was made exactly as the prechiasmatic cut except that the tip of the knife was placed 1 mm caudal to the bregma.

*Medial forebrain bundle lesions.* Bilateral electrolytic lesions were placed in the lateral hypothalamus to transect the fibers of the medial forebrain bundle as described previously[9].

*Midbrain raphe lesions.* The animals were placed in the stereotaxic apparatus with the tooth bar at 2.5 mm below the ear bars. The coordinates were anterior 2.0 mm, lateral zero, ventral 1.5 mm above the ear bars and at ear bar zero. At each point anodal DC current of 2 mA was passed for 30 sec.

*Lateral midbrain tegmentum lesions.* The animals were placed in the stereotaxic apparatus as in the group above. The coordinates were anterior 2.0 mm, lateral 1.2 mm from the midline on each side and ventral 1.5 mm and 0.5 mm above the ear bar zero. At each point anodal DC current of 2 mA was passed for 20 sec.

*Fornix lesions.* With the animals in the stereotaxic apparatus bilateral burr holes were placed extending from the coronal suture caudally for approximately 2 mm. The dura was incised and the cortex overlying the dorsal hippocampus aspirated through a fine, glass pipette to expose that structure. The hippocampus and fornix were then carefully aspirated bilaterally taking care not to involve either the underlying thalamus or the septum.

*Sham operation.* The 3 groups of sham operated animals were prepared. Each was done with a group of operated animals in order to provide this control with each set of operative procedures. For sham operation the animal was anesthetized, placed in the stereotaxic machine, and the skin over the skull was incised. Bilateral burr holes were placed in the parietal bone and these extended across with wound clips.

*Enzyme analysis.* Pineal serotonin N-acetyltransferase was analyzed by the method of Klein and Weller[14]. Each gland was thawed and then rapidly homogenized in 20 $\mu$l of 0.1 *M* sodium phosphate buffer, pH 6.8. In the initial experiments this contained 10 nmoles of [3′-$^{14}$C]serotonin (28 $\mu$Ci/$\mu$mole) and 20 nmoles acetyl coenzyme A. In subsequent experiments 20 nmoles of [$^{14}$C]serotonin and 40 nmoles acetyl coenzyme A were used. The reaction mixture was incubated for 10 min at 37 °C and the reaction was terminated by the addition of 20 $\mu$l of an ethanol–HCl solution (1:1, v/v) containing 20 nmoles of N-acetylserotonin and 20 nmoles melatonin. The samples were then centrifuged for 2 min at 18,000–20,000 $\times$ *g*. The [$^{14}$C]melatonin and [$^{14}$C]N-acetylserotonin in the supernatant were determined by liquid scintillation spectrometry following isolation by two dimensional thin-layer chromatography on precoated silica gel plates (initial development with chloroform: methanol:acetic acid, 90:10:1 by vol. followed by development with ethyl acetate in the second dimension). Enzyme activity was calculated for each gland as nanomoles of product formed per gland per hour.

Each point on the graphs (Figs. 6–8) illustrating the enzyme data represents the

mean ± S.E.M. for that point. At least 6 determinations were used for the mean for each point in each group except for the medial forebrain bundle and combined primary and accessory optic tract groups. Some of the points for those groups obtained during the light period are means of either 4 or 5 determinations.

## RESULTS

### *Lesion localization*

The location of all electrolytic lesions was verified by histologic study. That of the knife cuts was determined, in most instances, by gross inspection of the brain and sectioning the fixed brain with a razor blade. Several brains with knife cuts were examined histologically as well but this did not add greatly to the information obtained by gross inspection. A description of each lesion type follows. A diagrammatic representation of the location of each of the visual pathway lesions in the visual pathways is shown in Fig. 1.

*Accessory optic tract lesions.* The optic tract leaving the chiasm was destroyed in each case by a large cavitary lesion extending beyond the optic tract into the adjacent piriform cortex, medial and lateral hypothalamus and basal ganglia. The amount of lesion extension was variable but in most it was considerable. The contralateral optic tract exhibited substantial gliosis. In this group, both components of the accessory optic system, the superior and inferior fasciculi, were transected bilaterally. Both crossed components of the primary optic tracts were also ablated but the uncrossed component was preserved on one side.

*Primary optic tract lesions.* The lesions in these animals transected the primary optic tracts bilaterally at their entry into the thalamus and ablated the lateral geniculate nuclei totally extending to varying amounts into adjacent thalamus, subthalamus, pretectal area, tectum and hippocampus. The appearance of these lesions has been shown previously[21,22]. The animals had fixed, dilated pupils and exhibited no evident visual response to environmental stimuli.

*Primary and accessory optic tract lesions.* In this group one lesion transected the

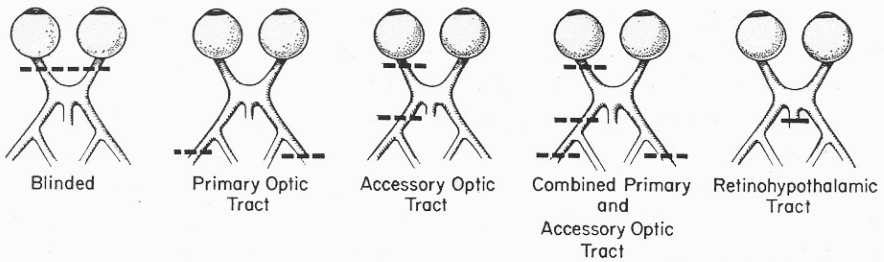


Fig. 1. Diagram of localization of visual pathway lesions. In blinded animals both optic nerves were cut by removing the eyes. Primary optic tract section was accomplished by bilateral lesions at the level of the lateral geniculate body. Accessory optic tract section was done by a unilateral ablation of all fibers leaving the chiasm and removing the eye on the same side, to remove the accessory optic tracts to the contralateral side of the brain. Primary and accessory optic tract destruction was accomplished by continuing these lesions. Retinohypothalamic tract section was made by ablation of both suprachiasmatic nuclei (see Figs. 2–4).

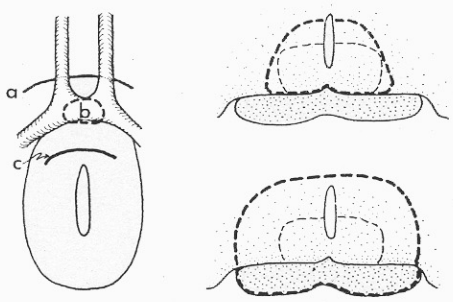


Fig. 2. Diagram showing location and size of hypothalamic knife cuts and suprachiasmatic lesions. The location of the prechiasmatic cut is shown by line a as it would appear on the ventral surface of the brain. The suprachiasmatic lesions were placed above the chiasm at b and the position of the postchiasmatic knife cut is shown by line c. The adjacent outline drawings depict the relative size of a small suprachiasmatic lesion as seen in a frontal section through the center of the nucleus at the top (lesion area outlined by dashed line) and a large lesion at the bottom.

primary and accessory optic tracts on one side at their emergence from the chiasm. Removal of the ipsilateral eye eliminated the contralateral accessory optic fibers and a contralateral lesion in the region of the lateral geniculate, as described above, removed the remaining primary optic tract fibers. These animals, thus, had no central retinal projections reaching terminal nuclei beyond the optic chiasm. As with the previous group, they showed no evident behavioral response to visual stimuli.

*Prechiasmatic cut.* The appearance of this lesion on the ventral surface of the brain is shown in Fig. 2. The tip of the knife passed through the septal area and olfactory tubercle rostral to the optic chiasm, sparing the optic nerves. Within the substance of the brain the lesion appeared as a thin necrotic region surrounded by reactive gliosis. Occasionally there were small hemorrhagic areas adjacent to the cuts.

*Postchiasmatic cut.* The appearance of this lesion on the surface of the brain is shown in Fig. 2. The tip of the knife passed through the tuberal hypothalamic area between the chiasm and the median eminence. As with the prechiasmatic lesion it appeared as a thin necrotic zone surrounded by gliosis. The location of the lesion was quite consistent.

*Suprachiasmatic lesions.* The lesions in animals prepared for the suprachiasmatic group fell into 3 distinct categories upon histological analysis and the data was originally analyzed upon this basis. The first of these were lesions in which the suprachiasmatic nuclei were ablated totally and bilaterally but in which the optic chiasm was partially spared. Examples of such lesions are shown in Figs. 3 and 4. In each case there was incidental damage to adjacent nuclei of the anterior hypothalamus but this was quite variable. The range of size of these lesions and their location projected onto the ventral surface of the brain is shown in Fig. 2. Some suprachiasmatic lesions extended through the optic chiasm to transect it but, as the enzyme data on such animals did not vary from those in which the chiasm was spared, they are included in the group. Animals in which the chiasm was spared, however, predominated at each point, particularly the one obtained for 2400 h. Such animals showed normal pupillary reactions and behavioral responses whereas those in which the chiasm was sectioned were like

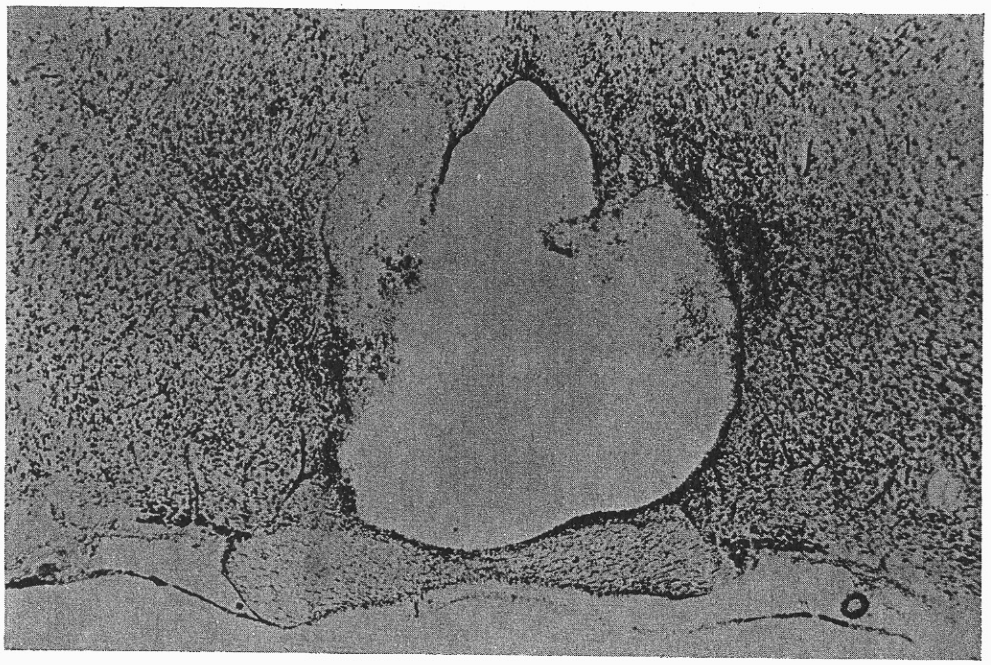

Fig. 3. Photomicrograph of a frontal section through a small suprachiasmatic lesion bilaterally ablating the suprachiasmatic nuclei but sparing the chiasm. The lesion extends slightly into the periventricular and anterior hypothalamic area adjacent to the 3rd ventricle. Cresyl violet stain, × 24.

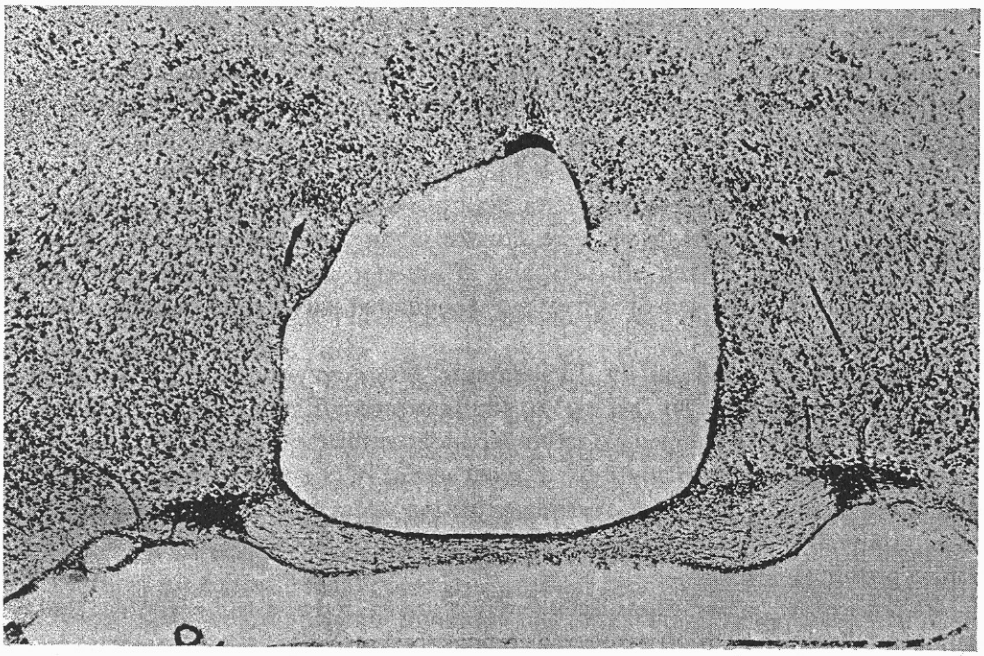

Fig. 4. Photomicrograph of a frontal section through a suprachiasmatic lesion bilaterally destroying those nuclei but extending widely into adjacent hypothalamus. This lesion also spares most of the optic chiasm fibers. Cresyl violet stain, × 24.

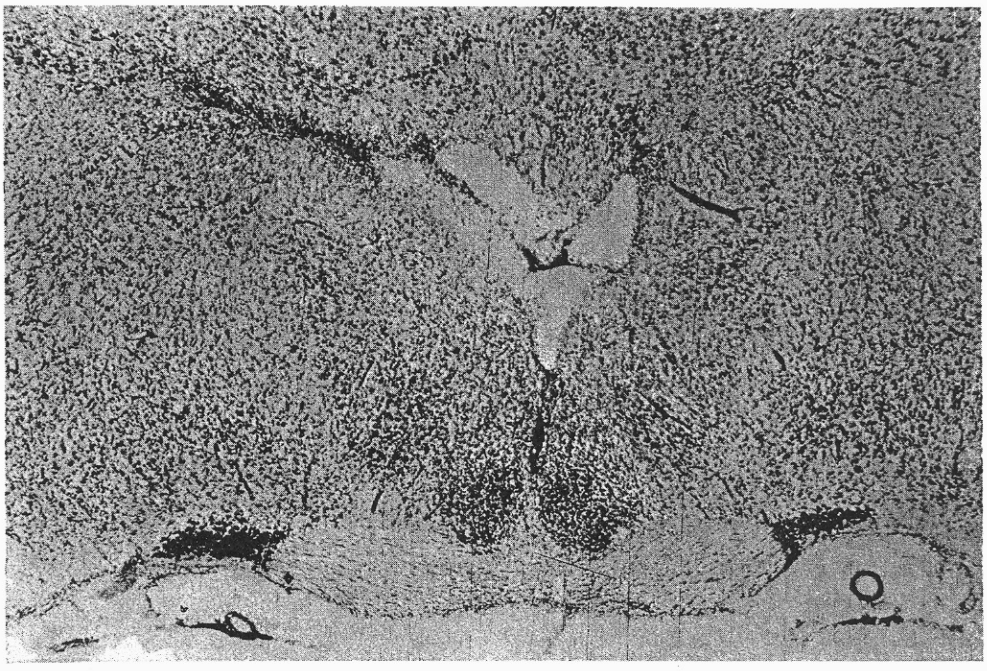

Fig. 5. Photomicrograph of a frontal section through an anterior hypothalamic lesion destroying tissue in the dorsal and medial hypothalamus above the suprachiasmatic nucleus. Cresyl violet stain, × 24.

the primary optic tract section group. The third category consisted of animals in which the suprachiasmatic lesion spared one or both of those nuclei and these were placed in another group described below.

*Anterior hypothalamic lesions.* The lesions in this group were intended to ablate the suprachiasmatic nuclei but were quite variably placed in the anterior hypothalamus in the vicinity of those nuclei. Most were situated in the midline dorsal to the suprachiasmatic nuclei (Fig. 5) and ablated parts of anterior and dorsal hypothalamic nuclei. Some were located laterally and extended somewhat into the lateral hypothalamus on one side.

*Medial forebrain bundle lesions.* The lesions in this group were located bilaterally in the lateral hypothalamus at the level of the ventromedial hypothalamic nucleus. All completely transected the medial forebrain bundle within the lateral hypothalamus and extended somewhat medially into adjacent nuclei of the tuberal region of the medial hypothalamus. There was variable extension dorsally into the subthalamic region and laterally into internal capsule. The appearance of these lesions is as has been described previously[9,21,22].

*Midbrain raphe lesions.* These lesions were centered in the rostral portion of the nucleus centralis superior of the raphe and completely destroyed that nucleus, extending variably into the adjacent dorsal raphe nucleus and laterally into nucleus pontis centralis oralis.

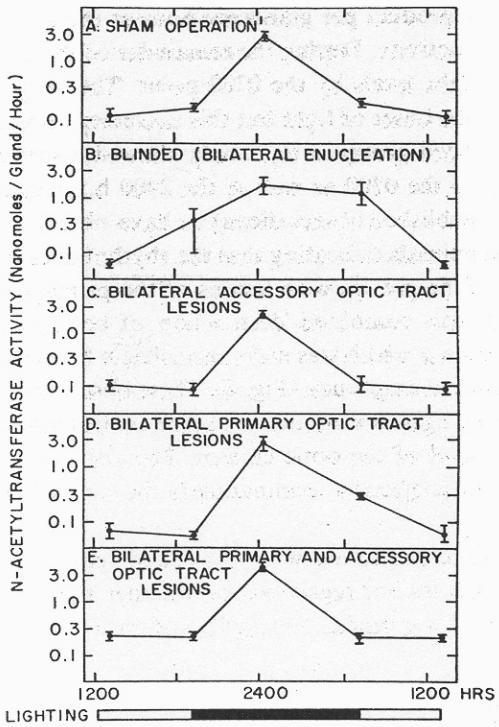


Fig. 6. Pineal N-acetyltransferase activity graphed for the sham operated, blinded, accessory optic tract, primary optic tract, and combined primary and accessory optic tract groups. In each case the rhythm persists. The lines above and below each point represent the S.E.M. in this and Figs. 7 and 8.

*Lateral tegmentum lesions.* These were bilateral lesions located in the lateral midbrain tegmentum adjacent to the rostral part of the nucleus centralis superior. They destroyed a large part of the nucleus pontis centralis oralis lying between the nucleus centralis superior medially, the nuclei of the lateral lemniscus laterally, the brachium conjunctivum and cuneiform area dorsally and the pontine nuclei ventrally. Most lesions were quite symmetrical but occasional ones were not and extended variably into adjacent structures.

*Fornix lesions.* The lesions in this group were bilateral. An extensive ablation of sensorimotor cortex was present in each and the anterior portion of the hippocampal formation was totally removed. Some lesions extended into adjacent thalamus but this was variable and usually minor.

*Serotonin N-acetyltransferase rhythm.* The observations on the N-acetyltransferase rhythm for each experimental group are shown in Figs. 6–8. In each set of experiments a separate sham operated group was run. These show the typical appearance of the N-acetyltransferase rhythm in the normal animal maintained in a normal, diurnal lighting schedule. The enzyme levels (graphed as a semi-log function against time) were low during the light period with mean values in the range of 0.05–0.3 nmoles of product formed per gland per hour. During the dark period enzyme levels

rose rapidly to approximately 3 nmoles of product per gland per hour at the 2400 h point, a 10- to 60-fold increase in enzyme activity. During the remainder of the dark period the values fell to reach the usual light levels by the 0700 point. The enzyme activity is known to fall rapidly following the onset of light but this decrement occurs even in the absence of light cues. The blinded group in this study showed a similar rhythm but the peak had shifted to include the 0700 as well as the 2400 h point. In other experiments (Moore and Klein, unpublished observations) we have observed a more clearcut shift of the peak in blinded animals indicating that the rhythm does become free-running in this situation. All of the groups with lesions of the primary or accessory optic pathways, including that with combined destruction of both pathways, exhibited a rhythm in N-acetyltransferase which was indistinguishable from that of the sham operated group in both timing and amplitude (Fig. 6). These observations indicate that entrainment of the rhythm by light is mediated by some central retinal projection terminating prior to or at the level of the optic chiasm. The only known pathway doing so is the retinohypothalamic projection terminating in the suprachiasmatic nuclei[23].

Transection of the retinohypothalamic projection was only found feasible by destroying the suprachiasmatic nuclei. This lesion, regardless of whether the optic chiasm was significantly involved, abolished the pineal N-acetyltransferase rhythm;

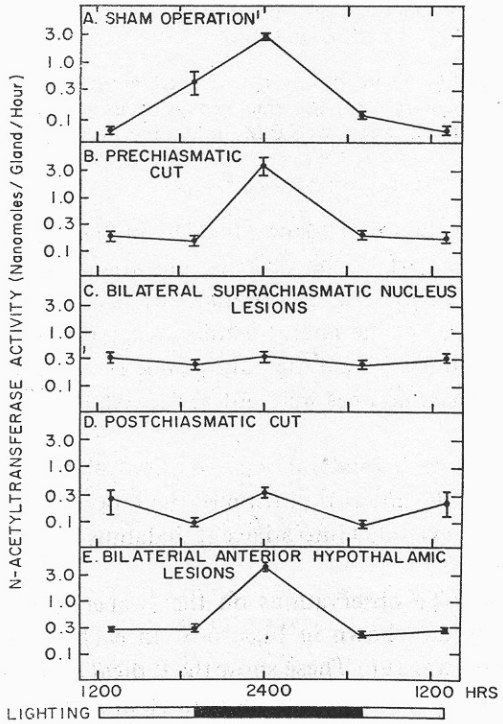


Fig. 7. Pineal N-acetyltransferase activity graphed for the sham operated, prechiasmatic, suprachiasmatic, postchiasmatic, and anterior hypothalamic groups. The rhythm is abolished in the suprachiasmatic lesion and postchiasmatic cut groups.

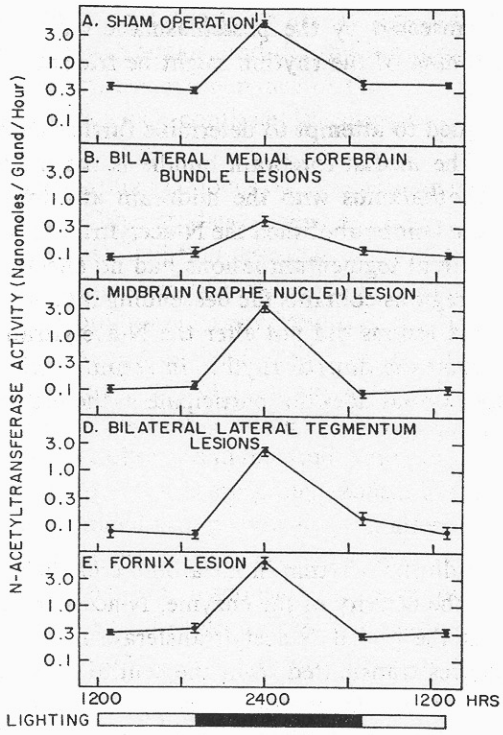


Fig. 8. Pineal N-acetyltransferase activity graphed for the sham operated, medial forebrain bundle, raphe lesion, lateral tegmentum lesion, and fornix lesion groups. The rhythm is abolished in the medial forebrain bundle group.

that is, no point sampled during the light or dark period differed significantly from any other point (Fig. 7). Lesions placed in the immediate vicinity of the suprachiasmatic nuclei, but sparing the nuclei, had no effect upon the rhythm indicating that nonspecific destruction of anterior hypothalamic tissue was insufficient to alter the rhythm. None of the suprachiasmatic lesions was completely restricted to the suprachiasmatic nuclei and it could be argued that involvement of some adjacent tissue or fibers passing through the region was responsible for the effects of this lesion. However, the histology of the lesions in this group indicated that no other tissue was systematically included in the lesion save for the periventricular zone immediately adjacent to the nuclei and part of the anterior hypothalamic area just dorsal to them.

The question of fibers of passage was experimentally examined in the prechiasmatic and postchiasmatic cut groups. The prechiasmatic cut did not alter the N-acetyltransferase rhythm. This indicates that fibers arising rostral to the suprachiasmatic region and passing through that region were not responsible for maintenance of endogenous control of the rhythm. Section of any such fibers would have been accomplished by the prechiasmatic cut. Postchiasmatic cuts, in contrast, produced effects which were identical to suprachiasmatic lesions: the N-acetyltransferase rhythm was abolished (Fig. 7). Fibers from suprachiasmatic nucleus neurons are known to pass

caudally[31] and these would have been transected by the postchiasmatic cut. This suggests that information regulating the timing of the rhythm might be transmitted caudally in this way.

The last set of experiments was designed to attempt to determine further pathways involved in regulating the rhythm. The medial forebrain bundle is the major fiber tract interconnecting the medial hypothalamus with the midbrain and lower brain stem. Bilateral medial forebrain bundle lesions abolished the N-acetyltransferase rhythm. The midbrain raphe lesions and lateral tegmentum lesions had no effect on the rhythm indicating that neither of these regions contains the descending influences regulating the timing of the rhythm. Fornix lesions did not alter the N-acetyltransferase rhythm, in contrast to their effect upon the diurnal rhythm in serum corticosterone content[17], indicating that the hippocampus does not participate in the regulation of the pineal N-acetyltransferase rhythm.

## DISCUSSION

The pineal gland in the rat exhibits a diurnal rhythm in melatonin content[16,24] which is controlled by a similar rhythm in the activity of the enzyme, N-acetyltransferase[12–14]. Prior studies have shown that the pineal N-acetyltransferase is a true circadian rhythm[12–14] regulated by influences transmitted from the central nervous system to the pineal[15] by its only known peripheral innervation, the superior cervical sympathetic nerves[11]. The central mechanisms controlling the pineal N-acetyltransferase rhythm appear to have 3 distinct components.

The first of these is the visual pathway transmitting information concerning environmental lighting to the brain. From the observations reported here, it would appear that the critical component of the central retinal projection for this function is a retinohypothalamic tract which terminates exclusively in the suprachiasmatic nuclei of the medial hypothalamus[10,18,23]. The existence of this pathway, so long in doubt in mammals, is now firmly established[10,18,23]. It has not been technically possible, however, to selectively section the retinohypothalamic tract and preserve the suprachiasmatic nuclei. For this reason, the conclusion that the retinohypothalamic tract mediates information critical to the synchronization of the pineal N-acetyltransferase rhythm is based on indirect evidence. That is, ablation of all the other known primary and accessory optic projections has no effect on the timing of the N-acetyltransferase rhythm so that, unless there is some other as yet unknown visual pathway, the retinohypothalamic tract is the only central retinal projection which could mediate the effects of light in controlling the rhythm.

The second component of the neural mechanisms regulating the pineal N-acetyltransferase rhythm is a central rhythm generator. The evidence that there is such a mechanism driving this rhythm is similar to that for other circadian rhythms[19]. The pineal N-acetyltransferase rhythm is precisely entrained in the normal animal by the pattern of environmental lighting. In the absence of lighting information, as in a blinded animal or one kept in continuous darkness, the rhythm continues but with a periodicity slightly variant from 24 h so that the rhythm peak drifts out of phase with a

24-h light cycle. Since there are no periodic visual cues reaching the brain in these light deprived animals, and the rhythm is out of phase with rhythms available in the environment, the maintenance of the rhythm must represent the function of central mechanisms which continue to produce rhythmic signals that influence the pineal gland.

The nature and locus of these driving mechanisms has only recently begun to be elucidated. Other studies, carried out largely concomitantly with those reported here, have demonstrated that destruction of the terminal nuclei of the retinohypothalamic projection, the suprachiasmatic nuclei, results in a loss of circadian rhythms in drinking and locomotor behavior[30] and in adrenal content of the corticoid, corticosterone, in the rat[20]. In each of these studies, as in the present experiments, the effect of suprachiasmatic nucleus ablation was a total loss of rhythmic function, not simply an alteration of the timing of the rhythm as would be expected from section of a visual pathway. We observed in this study that pineal N-acetyltransferase values in the suprachiasmatic nucleus lesion group remain low throughout a 24-h period whereas those from the blinded group show only an altered peak timing. This observation can only be explained by the interpretation that the suprachiasmatic lesion interfered with central mechanisms responsible for generation of the rhythm. Since the same lesion alters a variety of rhythms[20,30], it is suggested that the suprachiasmatic nuclei are a critical component of the central mechanism driving all these rhythms. It could be argued, however, that the lesions do not produce a specific effect but, rather, produce some general mutilative effects. There is nothing to support this concept. The animals recover very rapidly from the surgery, gain weight at the same rate as controls, and under gross observation exhibit normal cage behavior. This can be contrasted, for example, with the combined primary and accessory optic tract animals which have 3 large cerebral lesions. Half of the operated animals do not survive. The remaining animals recover slowly from the operation, losing weight during the initial week to 10 days, and are sluggish and inactive in their cages. They clearly show a general mutilative effect of brain lesions. However, at 21 days after operation these animals remarkably exhibit a normal pineal N-acetyltransferase rhythm.

Another question which arose is whether the loss of circadian rhythms could be attributed to destruction of neural elements other than the suprachiasmatic nuclei. This would not appear to be the case. The suprachiasmatic lesions are sufficiently variable that, by comparing large numbers of them, it is possible to exclude tissue lateral and dorsal to the nuclei as a consistent part of effective lesions. As noted above, and in a previous study[20], ablation of part or all of the chiasmal optic fibers does not contribute to the deficit. There is a small component of the periventricular hypothalamic nucleus uniformly destroyed in effective suprachiasmatic nucleus lesions and it is not possible to exclude this as a critical part of the lesion. The lesions also probably destroy fibers running from rostral to caudal within the medial hypothalamus but these, too, can be excluded from consideration because a knife cut placed just rostral to the suprachiasmatic nuclei has no effect on the N-acetyltransferase rhythm. All of these considerations strongly suggest that the suprachiasmatic nuclei function as a central rhythm generator, or oscillator, and this would appear to represent an appropriate

organization for the system since these nuclei receive the primary sensory input. It is not known, though, whether animals can compensate for the loss of this center with restoration of circadian functions. Thus far, our studies have been carried out at only 21 days after operation. Stephan and Zucker[30] observed animals for 3 months without noting recovery but longer term studies are obviously required before definitive conclusions about the permanence of the effect are drawn.

The third component of the central neural mechanisms regulating the pineal N-acetyltransferase rhythm is descending pathways from the suprachiasmatic nucleus to the intermediolateral cell column of the upper thoracic spinal cord from which the sympathetic innervation to the pineal arises. The work of Kappers[11] established the details of the peripheral sympathetic innervation of the pineal in the rat and the functional importance of this innervation was shown by Klein *et al.*[15]. From the present study the role of the retinohypothalamic projection and the suprachiasmatic nuclei has been emphasized. The efferent projection of suprachiasmatic nucleus neurons is not known and the only information available[31] suggests that they project caudally

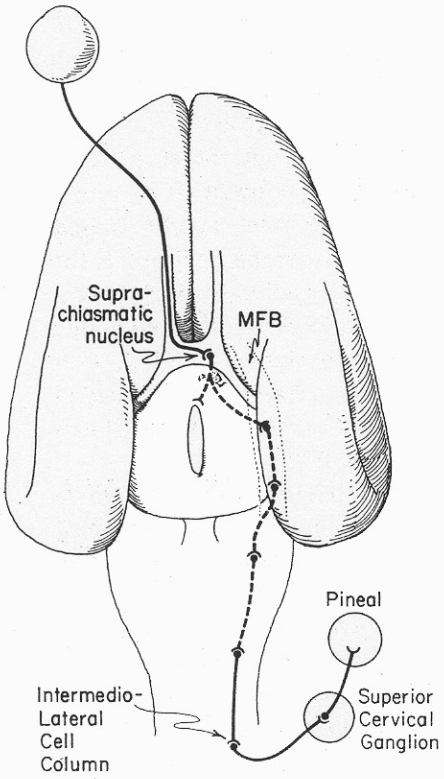


Fig. 9. Diagram of proposed central neural pathway regulating pineal N-acetyltransferase activity. Photic input from the eyes traverses the retinohypothalamic tracts to the suprachiasmatic nuclei. From these nuclei information passes through the lateral hypothalamus and brain stem, in unidentified pathways, to the spinal cord and the interomediolateral cell column whose neurons provide preganglionic fibers to the superior cervical ganglia. The dashed lines indicate areas where the exact fibers transmitting information have not been identified with certainty.

into the medial hypothalamus. Some corroboration for this is obtained from our observation that postchiasmatic knife cuts are identical in effect to suprachiasmatic nucleus ablation in abolishing the pineal N-acetyltransferase rhythm but we do not know where axons of suprachiasmatic nucleus neurons terminate. Similar knife cuts abolish pituitary–adrenal rhythms[8,20] in rats and block the effect of constant darkness in the production of a pineal-induced gonadal atrophy in the hamster[27]. Presumably there is a multisynaptic pathway to the medullary reticular formation (Fig. 9) with a reticulospinal projection finally reaching the thoracic cord but this is only speculative at present. This pathway may well involve descending fibers within the medial forebrain bundle since bilateral destruction of this tract also abolishes the N-acetyltransferase rhythm, a pineal response to olfactory bulb ablation and blinding[28], and a rhythm in hepatic tyrosine transaminase activity that is probably regulated by sympathetic input[5]. This cannot be accepted without reservation, however, because bilateral lesions of the medial forebrain bundle have marked effects on eating, drinking, and activity and are certainly mutilative in a general sense. None of the tegmental lesions, either in the midline raphe nuclei or in the lateral midbrain tegmentum, altered the N-acetyltransferase rhythm indicating that the descending pathways are not exclusively located in either of these regions. Similarly, transection of the fornix was without effect on the rhythm so that descending hippocampal influences, which may affect the adrenal corticosterone rhythm[17], can be excluded as contributors to regulation of the N-acetyltransferase rhythm.

A final point to be considered is the relationship between visual pathways controlling pineal N-acetyltransferase activity in the rat and those controlling the pineal hydroxyindole-O-methyltransferase (HIOMT) response to light. Unlike N-acetyltransferase, HIOMT does not appear to consistently exhibit a significant diurnal rhythm[12]. In animals placed in continuous darkness or blinded, however, pineal HIOMT levels are approximately twice those observed in animals maintained in diurnal or continuous light[12,21]. Prior studies have indicated that the pineal HIOMT response to light is mediated by the accessory optic system[21,22]. That is, transection of the inferior accessory optic tract in the rat is equivalent to blinding in that it results in continuously high pineal HIOMT levels that are independent of environmental lighting conditions. This separation of function is supported by observations that transection of the primary optic tracts[21,22] or destruction of the suprachiasmatic nuclei (Moore, unpublished observations) does not affect the pineal HIOMT response to light. This would support the concept, first put forth by Stephan and Zucker[29], that the accessory optic system mediates tonic, or continuous, effects of light whereas the retinohypothalamic projection mediates rhythmic, or phasic, effects and the primary optic system mediates information concerning the organization of the visual environment as this is used in behavioral responses[19]. This requires further testing and analysis. Critchlow[6] has shown, for example, that constant vaginal estrous responses to continuous light in the female rat are blocked by lesions in the suprachiasmatic region. The exact localization of the lesions is not described but they appear comparable to the frontal knife cuts reported by Halasz[7] and Moore and Eichler[20] to affect pituitary function. These observations, and the data reported here, suggest that the visual

pathways regulating pituitary function and those regulating pineal function separate in the medial hypothalamus caudal to the suprachiasmatic region. The pathways controlling the pituitary would be directed toward the median eminence and those controlling the pineal into the descending component of the medial forebrain bundle. The exact location of these pathways remains to be determined by further experiments.

As noted above, the pineal gland is an active site of indoleamine metabolism[12]. A principal product of this metabolism is the antigonadal substance, melatonin. The production of melatonin in the pineal is regulated by the rate-controlling step in the biosynthetic pathway, the conversion of serotonin to N-acetylserotonin by N-acetyltransferase[12]. The activity of this enzyme in the pineal gland exhibits a remarkable circadian rhythm[14] which is controlled by visual input and a central generating mechanism. The results of the present study, summarized in Fig. 9, have demonstrated some of the components of this system; others remain to be elucidated. These observations are pertinent not only to our understanding of the regulation of pineal function but to that of the central control of rhythmic events in mammalian organisms.

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